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<b>(71) Applicant (for all designated States except US):</b> ANRET LTD. [BG/BG]; Dragan Tsankov Boulevard, Bl.59-63, Entr.B, fl.2, Ap.4, 1172 Sofia (BG).			
<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> GABEV, Evgeni Bognilov [BG/BG]; Acad. Metody Popov Street 10, Bl.63, 1113 Sofia (BG). GABEV, Evgeni Evgeniev [BG/BG]; kv.Dianabad, Bl.41, fl.11, Ap.55, 1172 Sofia (BG).			
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**(54) Title:** LIPOSOMES COMPRISING AZIDOTHYMIDINE AND LITHIUM FOR TREATING HIV/AIDS

**(57) Abstract**

The invention relates to a composition for treating HIV/AIDS. The composition, subject of the present invention shows increased anti HIV activity, has a depot effect and it is non toxic. Also due to increased intervals of administration and reduced doses the treating costs are lower in comparison to the routine anti HIV drugs. The composition is a liposome encapsulated combination of Azidothymidine and Lithium ions ( $Li^+$ ) in w/w ratio from 30:1 to 20:1.

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**LIPOSOMES COMPRISING AZIDOTHYMIDINE AND LITHIUM FOR TREATING HIV/AIDS****FIELD OF TECHNICS**

The invention concerns the field of drugs for treating of HIV/AIDS.

**BACKGROUND OF THE TECHNICS**

At present for treating of HIV/AIDS three basic groups of preparations are used:

- a) nucleoside inhibitors of reverse viral transcriptase.
- b) non nucleoside inhibitors of reverse viral transcriptase.
- c) inhibitors of the viral proteinase.

From the nucleoside inhibitors group the Azidothymidine (with generic name zidovudine known as well as AZT) is the first and generally accepted drug. Dideoxycytidine (ddC) and didanosine (ddI) are also used. 3TC and d4T preparations are still in clinical trial.

The Nevirapine preparation is from the group of the non nucleoside inhibitors of reverse viral transcriptase, which is in the stage of clinical trial.

Three preparations from the group of the inhibitors of viral proteinase, which are applied in widened clinical trials are approved in some countries for clinical application: Saquinavir, Ritonavir, Indinavir.

All these anti-HIV drugs have restricted clinical application for treating HIV/AIDS, because of the following disadvantages and side effects:

- a) neither of the preparations lead to curing of the disease, but only temporary improve the condition of the patient, while after ceasing their application the disease is resumed.
- b) high toxicity, which necessitates the untimely ceasing of the treatment.
- c) the preparations lead to the creation of resistance of HIV towards them which compromises their further use.
- d) they have no effect on the latent form of HIV (the proviral DNA in the reservoirs of the virus).
- e) extremely high price, which makes the treatment difficult for mass application.

In recent years widely is maintained the attitude of treating HIV/AIDS with combinations of different groups of preparations, acting with different mechanism with a view to overcome the disadvantages of the monotherapy, and in such medicine combinations AZT is preferred (1).

On the other hand during recent years the liposome drug delivery have wider practical application. Considering the accompanying AIDS opportunistic infections and tumor diseases, such liposomal preparations are AmBisome (2),

which is a liposomal form of amphotericin B for treatment of candidosis and cryptococcosis and DaunoXome (3), a liposomal preparation of Daunorubicin for treatment of Kaposi's sarcoma.

Till now a liposomal preparation with direct anti-HIV action is not known in practice.

## SUMMARY OF THE INVENTION

The present invention offers composition for treating of HIV/AIDS, containing Azidothymidine and Lithium ( $\text{Li}^+$ ) ions in ratio from 30:1 to 20:1 w/w, encapsulated in liposomes (lipid vesicles).

It is offered that the composition should be administered intravenously and/or rectally in a dose regarding AZT from 1 to 10 mg/kg body mass once weekly or one time every two weeks depending on the clinical laboratory tests results and the stage of the diseases.

Major advantages of the composition, subject of the present invention, compared to the drugs used till now are:

- a) increased anti-HIV activity (more than 65 times in comparison to AZT), determined *in vitro*.
- b) absence of toxicity.
- c) depot effect.

Not lesser are the following advantages:

- a) increasing of the intervals of administration of the composition to once weekly or one time every two weeks.
- b) strong reduction of the doses regarding the routinely applied doses of AZT.
- c) stimulation of the leucopoisis and the regeneration of the hepatocytes.
- d) high penetration ability through the cell membranes.
- e) lower prime cost in comparison to the used anti-HIV preparations.

## EMBODIMENTS OF THE INVENTION

Example 1. A composition according to the invention, containing AZT and Lithium ions in ratio 30:1 w/w, encapsulated in liposomes (lipid vesicles), AZT is encapsulated in the lipid phase, and the Lithium ions in the aqueous phase, being 0.9% aqueous solution of sodium chloride.

Example 2. A composition according to the invention, containing AZT and Lithium ions in ratio 25:1 w/w, encapsulated in liposomes (lipid vesicles), AZT is encapsulated in the lipid phase and the Lithium ions in the aqueous phase, being 0.9% aqueous solution of sodium chloride.

Example 3. A composition according to the invention, containing AZT and Lithium ions in ratio 20:1 w/w, encapsulated in liposomes (lipid vesicles),

AZT is encapsulated in the lipid phase and the Lithium ions in the aqueous phase, being 0.9% aqueous solution of sodium chloride.

Example 4. Comparative investigation of the composition, subject of the invention for anti-HIV efficacy and cytotoxicity *in vitro*.

The composition subject of the invention was investigated for anti-HIV efficacy on cell cultures not infected with the virus of AIDS, and for cytotoxicity on not infected cultures of the same kind, in comparison with non encapsulated in liposomes (free) AZT under equal experimental conditions and doses. The efficacy was determined by measuring the quantity of the p24 antigen specific for HIV by the ELISA and the cytotoxicity was determined spectrophotometrically with MTT. The results of the above mentioned investigation showed 67 times average higher anti-HIV efficacy in comparison to the non liposome encapsulated (free) AZT and absence of cytotoxicity.

Example 5. A comparative investigation of acute toxicity *in vivo*. The composition, subject of the invention and a mixture of AZT and Lithium ions non encapsulated in liposomes (free) were investigated for acute toxicity on experimental animals (rats and mice) from both sexes, by intravenous and subcutaneous mode of administration in a single dose 100 times higher than the proposed AZT curative dose. A separate group of animals were injected with sodium saline, which was used as a control group. The indices for acute toxicity were monitored for 1 month post injection. The results showed absence of acute toxicity of the composition subject of the invention.

Example 6. A comparative investigation of chronic toxicity *in vivo*. The investigation of chronic toxicity was carried out for 7 months on experimental animals (rats) from both sexes by intravenous administration at intervals of one week for 6 months with the proposed curative dose and ten times the curative dose. The composition and a mixture of AZT and Lithium ions non encapsulated in liposomes (free) under equal experimental conditions and doses were investigated according to the invention. A separate group of animals injected with sodium saline was used as a control group. A number of indices were monitored according to the requirements for carrying out chronic toxicity including hematological, clinical, biochemical and pathoanatomical indices at the end of the first, the third, the sixth and the seventh month. The results of the investigation showed absence of chronic toxicity of the composition subject of the invention. The electron microscopy data indicate stimulation of the leucopoiesis and the regeneration of the hepatocytes of the treated animals.

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2. Hay R. J. Liposomal amphotericine B, AmBisome. *J Infect* 28 Suppl. 1(1994 May, pg. 35-43.
3. Chew T., Jacobs M., Huckabee M., Ross M. A phase II clinical trial of DaunoXome (VS103, liposomal daunorubicin) in Kaposi's sarcoma of AIDS patients. *Int. Conf. AIDS* 9(1), 1993 Jun 6-11, pg. 58 (abstract No. WS-B15-3).

**PATENT CLAIMS**

- 1) Composition for ~~treating~~ of HIV/AIDS, which contains a combination of Azidothymidine and Lithium ions ( $\text{Li}^+$ ) in w/w ratio from 30:1 to 20:1 encapsulated in liposomes (lipid vesicles).

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<b>(74) Agent:</b> HRISTOVA, Lilyana Lyubomirova; Gatev & Shentova Patent Bureau, Damyan Gruev Street 11, 1606 Sofia (BG).		<b>(88) Date of publication of the international search report:</b> 30 July 1998 (30.07.98)	

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## INTERNATIONAL SEARCH REPORT

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PCT/BG 97/00013A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/70 A61K33/00 A61K9/127 // (A61K31/70, 33:00)

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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>STN INTERNATIONAL, KARLSRUHE. FILE CHEMICAL ABSTRACTS, AN=122:150948, XP002065349 see abstract &amp; E. TOWNSLEY: "Lithium and anti-viral drug toxicity. I. Further studies on the ability of lithium to modulate the hematopoietic toxicity associated with the anti-viral drug zidovudine (AZT)" J. TRACE MICROPROBE TECH. (1995), 13(1), 1-9,</p> <p style="text-align: center;">-/-</p>	1

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Y	STN INTERNATIONAL, KARLSRUHE. FILE AIDSLINE, AN=1992:12195, XP002065350 see abstract & S. BERMAN: "Double-blind placebo controlled trial of the efficacy of lithium in treating neutropenia in HIV infected patients." INT CONF AIDS, (1992). VOL. 8, NO. 2, PP. B214, ---	1
Y	STN INTERNATIONAL, KARLSRUHE. FILE AIDSLINE, AN=1991:10969, XP002065351 see abstract & E.E. GABEV: "In vitro study of liposome encapsulated AZT and its combination with a presumptive enzyme blocking agent (PEBA)" INT. CONF. AIDS, (1991). VOL. 7, NO. 2, PP. 92, ---	1